Award Number: W81XWH-14-2-0153

TITLE: Decreasing Skin Graft Contraction through Topical Wound Bed Preparation with Anti-Inflammatory Agents

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REPORT DATE: OCT 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
October 2017	Annual	09/15/2016 - 09/14/2017

4. TITLE AND SUBTITLE
"Decreasing Skin Graft Contraction through Topical Wound Bed Preparation with Anti-Inflammatory Agents"

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-2-0153

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S Army Medical Research and Materiel Command
Ft Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The objectives of this proposal are to identify the dose and application schedule of a specific topical anti-inflammatory drug that will reduce and shorten the inflammatory state of the recipient wound bed and thus, skin graft contraction.

15. SUBJECT TERMS Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
			Unclassified	12	USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION

We hypothesize that the elevated and prolonged inflammatory state of the recipient wound bed is a causative factor in the development of skin graft contraction. Using a porcine model of skin graft contraction, we will screen for anti-inflammatory agents (dose, schedule of administration, drug class) that reduce inflammatory cytokines in the recipient wound bed during 6 days post-wounding. We will then validate the effectiveness of the anti-inflammatory agent, dose and schedule to reduce contraction of the grafted split-thickness skin by allowing the experimental animal to survive for a longer period of time. Specifically, the aims of the proposal are to develop treatments that modulate inflammation and decrease skin graft contraction. We will achieve this by (1) identifying a dose and schedule of anti-inflammatory drug that most effectively blocks excessive inflammation of the recipient wound bed as defined by inflammatory markers and (2) validate the schedule and dose of anti-inflammatory drug shown to reduce inflammation of the recipient wound bed to decrease skin graft contraction.

KEYWORDS

-Inflammation
-Anti-inflammatory agents
-Wound healing
-Contraction

ACCOMPLISHMENTS

What were the major goals of the project?
Develop treatments that modulate inflammation and decrease skin graft contraction

Aim 1: Identify a dose and schedule of anti-inflammatory drug that most effectively blocks excessive inflammation of the recipient wound bed as defined by inflammatory markers.

Aim 2: Validate the schedule and dose of anti-inflammatory drug shown to reduce inflammation of the recipient wound bed to decrease skin graft contraction. (Performed on a large wound model)

What was accomplished under these goals?

Aim 1: Swine Studies/Surgeries - Completed.

Progress: From the Aim 1 experiments we learned that application of a modulatory drug(s) prior to grafting (or underneath the split-thickness skin graft) universally led to graft failure. Knowing this we modified the treatment plan and applied the treatment after applying the skin graft. The animal experiments for Aim 1 have been completed. Graft take at 14 days was analyzed (Fig 1). The histology and quantification of inflammatory markers is currently being analyzed and is analyzed at the same time as tissues from Aim 2. Wound contraction was not a primary endpoint for Aim 1 and thus was not assessed. However, it is known that poor graft take likely results in more contraction.

Overall, based on both subjective observation and objective analysis using the Silhouette Star® we found that modulating the wound at day 3 (late treatment) lead to improved Split-Thickness Skin Graft (STSG) take when compared to applying immediately on day 0 (early treatment) in six of the nine treatment groups. However, no statistical significance between the groups can be made as the total number of each treatment group was low. Therefore, all drugs have been carried over and are being utilized in Aim 2.

Methods/Results: The four swine used in Aim 1 were split into two early and two late treatment groups. In the early group, excision, grafting and application of the modulatory drug all occurred on day 0. Although overall graft take was greater than 90% for all treatment groups, Indomethacin treated wounds had the worse graft survival (90.37%) at day 14 (Figure 1). Interestingly, other Indomethacin containing groups did not perform as poorly. In fact, the Indomethacin-Dexamethasone combination had the second highest graft survival rate if the control (STSG-only) is not considered.

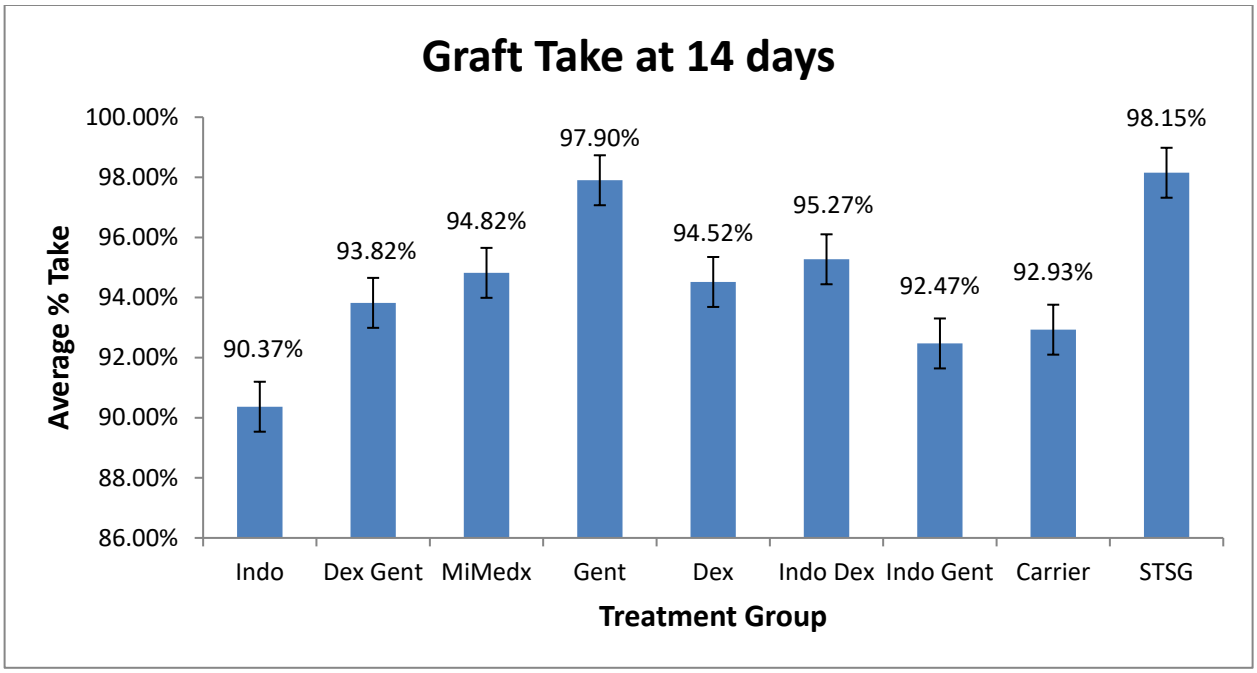

Figure 1: Average graft take at 14 days in the early treatment group

The late treatment groups differed from the early groups in that excision and grafting occurred on day 0. The wounds were then covered with an occlusive dressing and on day 3 the modulatory drug was applied. As shown in Figure 2, graft was worse in the Gentamicin containing groups, however, these differences are likely not significantly different.

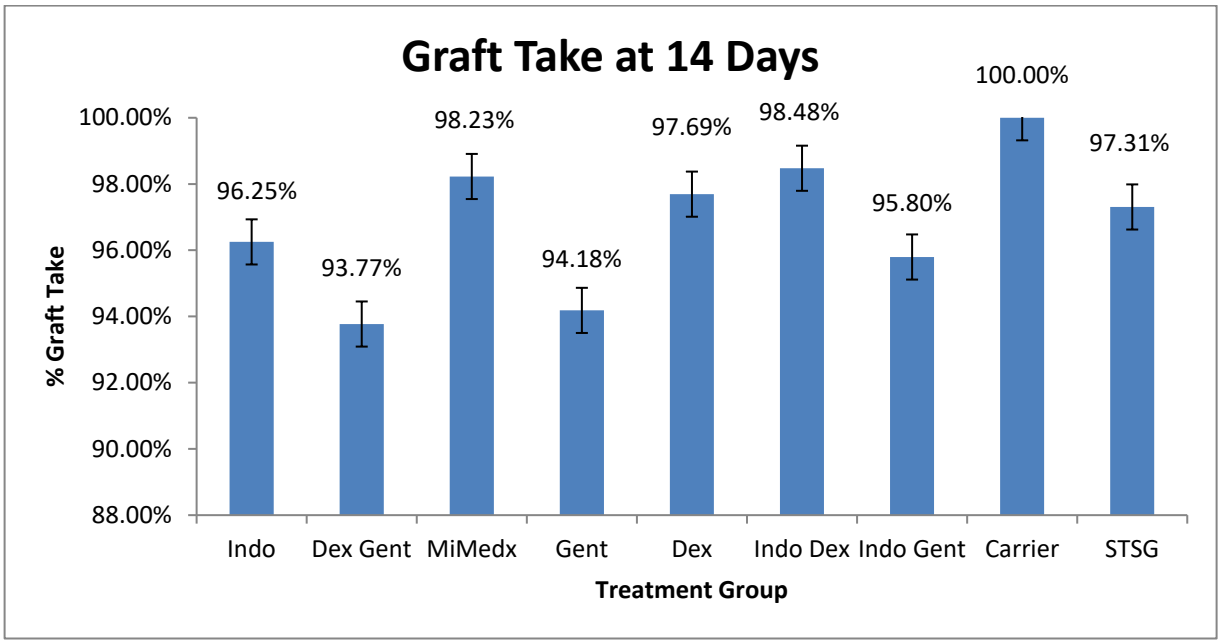

Figure 2: Average graft take at 14 days in the late treatment group

Despite the low number of overall wounds (n), when comparing the early and late treatment groups (Figure 3) the data demonstrates that overall graft take in both the early and late treatment groups exceeded 90% with the late treated groups having slightly better results.

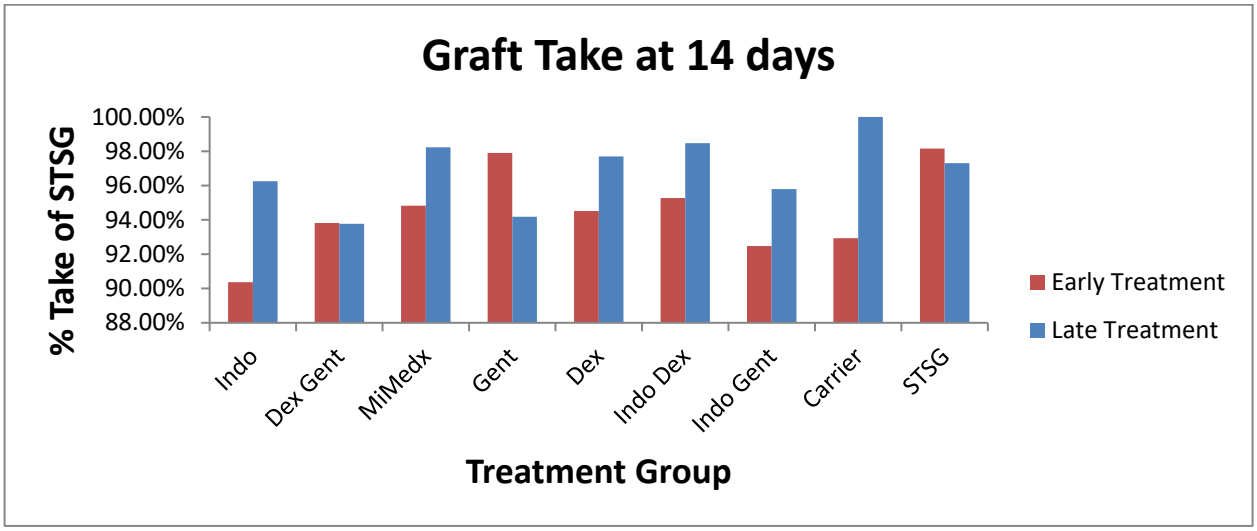

Figure 3: Comparison of average graft take at 14 days in the early and late treatment group

Furthermore, unlike prior experiments where other vehicles for medication delivery were utilized and led to poor results, the carrier (hydrogel) in these experiments led to >90% graft take. This confirms that a hydrogel is an appropriate carrier to be used in this study.

Based on the presented data, we feel confident that hydrogels are the appropriate carrier for delivery of the modulatory medications. Second, we believe that the current drug dosage is appropriate. Last, applying the modulatory drug late (day 3) provides the best overall results in six of the nine treatment groups. Of the three treatment groups that demonstrated improved take with early modulation, two (Dex/Gent and STSG) had very similar results differing by only 0.05% in the Dex/Gent group and 0.84 in the STSG group.

Our next step in Aim 1, while simultaneously performing Aim 2, is to complete histological analysis of the biopsied specimens and assess the affect the drugs have on wound bed inflammation.

Histological and cytokine analysis is currently being evaluated and occurs at the same time as tissue harvested during Aim 2.

Aim 2:
Progress: Aim 2 utilized larger wounds. In this aim, the wounds were circular with a 6-cm diameter. A total of 8 animal surgeries/experiments were planned for this aim with two additional replacement animals available in case of complications affecting the study results. To date, 6 out of the 8 animals have reached the endpoint and the remaining two animals have their endpoints next week (Oct 19).

In this aim, the modulatory drugs were applied on post-operative day 3. The decision to pursue late treatment only was based on the results from Aim 1. In Aim 1, six of the nine groups demonstrated improved graft take when comparing early vs. late treatment. Two of the remaining 3 groups (Dex/Gent and STSG) had comparable results with the early treatment. Therefore, the group consensus is that late treatment leads to improved outcomes. The endpoints for Aim 2 were; graft take, inflammatory modulation based on quantification of inflammatory markers, histology as well as wound contracture and scar outcomes at 120 days.

To date, all but two swine have completed the 120-day experiment. The data shown below are data from the six swine that have completed the experiment.

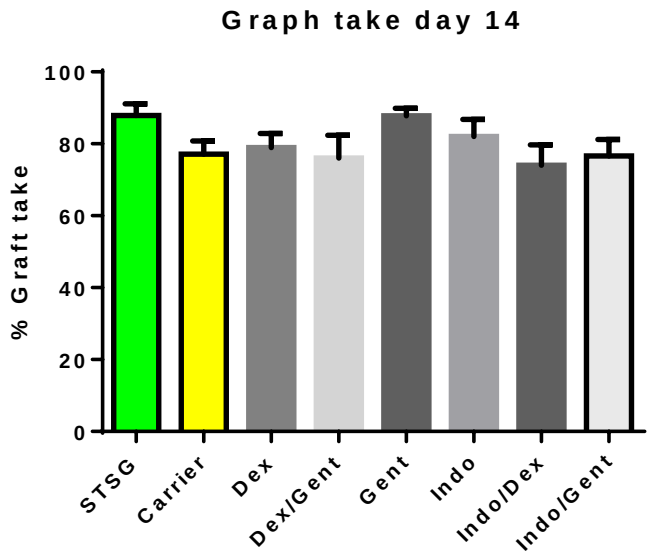

Figure 4: Average graft take at 14 days based on treatment groups

The change in area where measured throughout the course of the experiment using a Silhouette star device. The graphs below compare the wound size at day 3 with the scar at day 120. The data is shown both in % area change and % area change compared to the STSG control group.

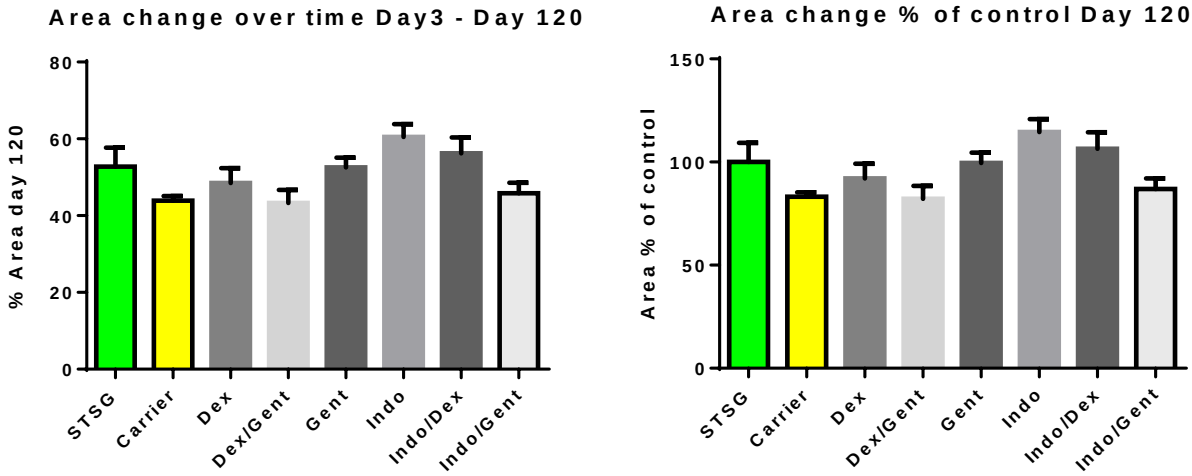

Figure 5: Left – Shows the decrease in area of the scars based on treatment group. Right – Shows the area change compared to STSG control.

Trans-epidermal water loss (TEWL) and Conductance are both measurements to determine skin barrier function and moisture content. The TEWL measure how much water evaporates over a determined area while the Conductance measures the electrical conductance of the skin.

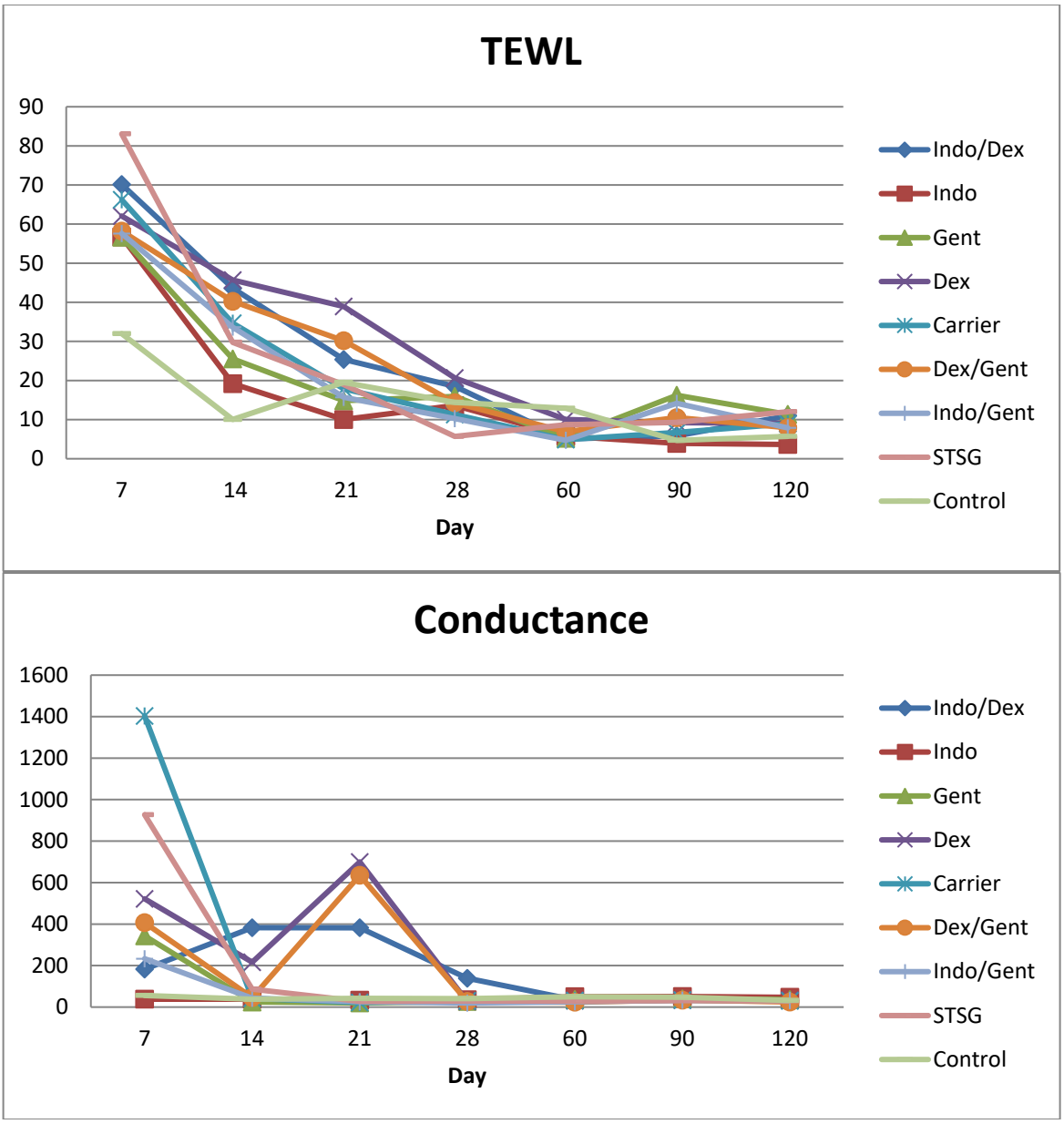

Figure 6: Top – Shows TEWL for each treatment group from day 7 to day 120. Bottom - Shows Conductance for each treatment group from day 7 to day 120.

Laser Speckle Contrast Analysis is a method that visualizes tissue blood perfusion in the microcirculation instantaneously. A Laser Speckle device from Perimed was used from day 7 to day 120. The figure below visualizes the data over time and treatment group.

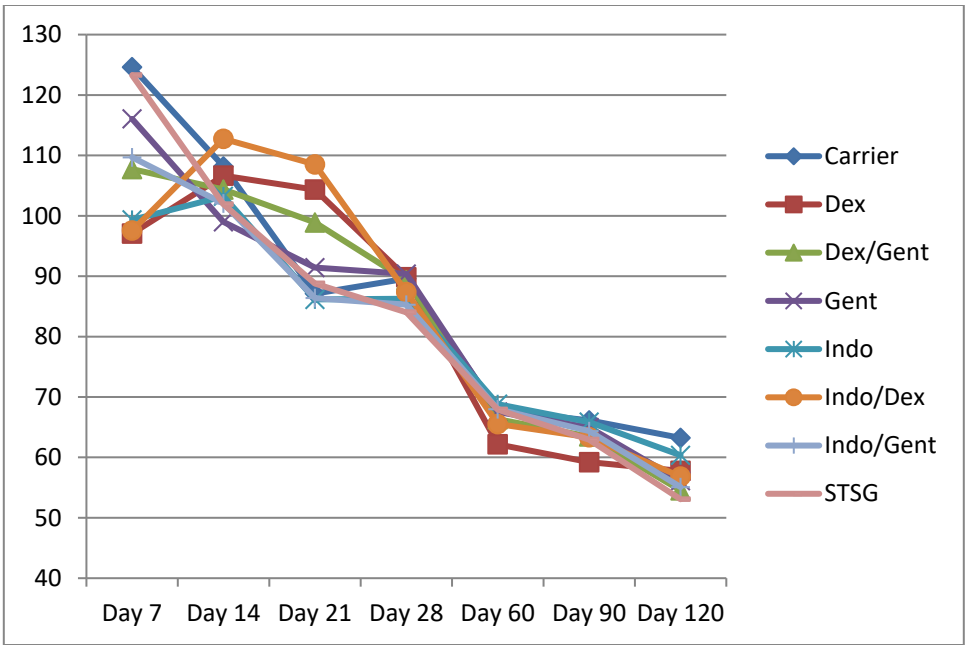

Figure 7: Laser speckle values over time based on treatment group.

The remaining steps include; 1) Inclusion of the last two animals in all data analysis, 2) Analysis of inflammatory markers using Luminex/bioplex, 3) Histopathological assessment and scoring.

What opportunities for training and professional development has the project provided?

This project has provided research training for post-doctoral fellows with study design and execution including harvesting skin grafts, applying split-thickness skin grafts to wounds and suturing grafts in place. The preliminary findings have been presented at local conferences but there have been no publications to date.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

The final steps of this study include:

- Adding data from the final two remaining animals to analysis and statistical assessment.
- Cytokine analysis from tissue biopsies using Luminex/Bioplex kit
- Receive histopathological assessment

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

In this project, we have learned that placement of anti-inflammatory modulators prior to a split-thickness skin graft inevitably leads to graft failure. This is likely due to the inhibition of angiogenesis and migration of essential nutrients to the graft.

What was the impact on other disciplines?

It is known that imbibition is vital for skin graft survival. Based on our results, we have demonstrated that application of a substance that limits imbibition has a profound effect on graft survival. This knowledge will likely prevent other scientists and clinicians from placing materials between a wound and skin graft and compromising the integrity or "take" of their graft.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS

Changes in approach and reasons for change

Old Approach: Placement of anti-inflammatory agent over wound bed followed by placement of skin graft. This led to graft failure.

New Approach: Placement of skin graft prior to applying anti-inflammatory agents. This is not a significant change and was discussed in the proposal.

Actual or anticipated problems or delays and actions or plans to resolve them

The delay in experiments is a result of the shortage of water-soluble dexamethasone – an issue stated in previous reports. The Dexamethasone was on backorder with the parent company and no suitable substitutes could be found. Other than the previous issue described above there is Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

No changes.

PRODUCTS

Publications, conference papers, and presentations

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Rodney Chan, MD.
Project Role:	Principal Investigator
Nearest person month worked:	6
Contribution to Project:	Dr. Chan is the PI of the award.

Name:	Kai Leung, PhD.
Project Role:	Co-Principal Investigator
Nearest person month worked:	.0012
Contribution to Project:	Dr. Leung is the Co-PI of the award.

Name:	Anders Carlsson, PhD.
Project Role:	Post-Doctorate
Nearest person month worked:	1.8
Contribution to Project:	Dr. Carlsson is a Post-Doctorate assisting with completion of this project.

Name:	Remington Wong
Project Role:	Research Technician
Nearest person month worked:	0.6
Contribution to Project:	Mr. Wong is the Research Technician of this award.

Name:	Chris Corkins, M.D.
Project Role:	Surgical Resident
Nearest person month worked:	2.4
Contribution to Project:	Dr. Corkins is a Surgical Resident assisting with this project.

Name:	John Fletcher, M.D.
Project Role:	Surgical Resident
Nearest person month worked:	0.12
Contribution to Project:	Dr. Fletcher is a Surgical Resident assisting with this project.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

SPECIAL REPORTING REQUIREMENTS

Quad Chart: The Quad is updated and submitted as an appendix.

APPENDICES

N/A

Decreasing skin graft contraction through topical wound bed preparation with anti-inflammatory agents

W81XWH-14-2-0153

PI: Rodney Chan MD/Kai Leung PhD **Org:** USAISR/The Geneva Foundation **Award Amount:** $881,310

Study/Product Aim(s)

1. Identify a dose and schedule of anti-inflammatory drug that most effectively blocks excessive inflammation of the recipient wound bed as defined by inflammatory markers.
2. Validate the dose and schedule of anti-inflammatory drug shown to reduce inflammation of the recipient wound bed to decrease skin graft contraction in a large wound model.

Approach

A porcine model of excisional wound was developed to study wound inflammation and its effect on skin graft contraction. Wound bed modulation using anti-inflammatory treatments are first applied to a screening model and then validated on an experimental model with larger wounds to study skin graft contraction.

Aim 2 (Data based on 6/8 swine experiments):

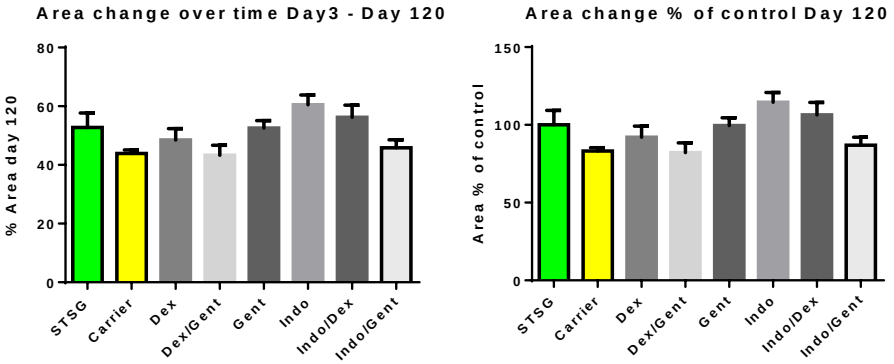

Figure 5: Left – Shows the decrease in area of the scars based on treatment group. Right – Shows the area change compared to STSG control.

Timeline and Cost

Activities CY	14	15	16	17
Aim 1 (Identify drug/schedule/dose) using screening model				
Aim 2 (Identify drug/schedule/dose) using validation model				
Complete Data Analysis				
Manuscript				
Estimated Budget ($881.3K)	**$0**	**$434**	**$447**	**$0**

Updated: 12 Oct2017

Goals/Milestones

CY15/16 Goals – Screening of anti-inflammatory therapies

- ☑ IRB Approval of both screening and validation porcine wound bed preparation model
- ☑ Establishment of Validation model to examine the effect of topical anti-inflammatory drugs
- ☑ Establishment of Screening model to examine the effect of topical anti-inflammatory drugs
- ☑ Establish dose and schedule of anti-inflammatory drug best to decrease inflammatory markers (completed but additional follow on screening added)

CY16/17 Goals – Validation of anti-inflammatory therapies

- ☑ Establish dose and schedule of anti-inflammatory drug best to decrease inflammatory markers
- ☑ Validate the optimal dose and schedule of anti-inflammatory drug to decrease skin graft contraction in a large wound model
- ❑ Complete Data Analysis
- ❑ Draft Manuscript

Comments/Challenges/Issues/Concerns: Late delivery of water-soluble dexamethasone for the Dex containing groups resulted in a delay of experiments during aim 2. The endpoint of the final pair of animals is week 42.

Budget Expenditure as of
Projected Expenditure: $881,310
Actual Expenditure: $791,974